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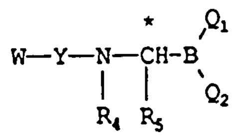
(57) Abstract

Peptidomimetics of formula (I), wherein W, Y, R_4, R_5, Q_1 and Q_2 are defined in claim 1 are potent thrombin inhibitors.



(I)

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PEPTIDE BORONIC ACID DERIVATIVES HAVING PROTEASE INHIBITING ACTIVITY

This invention relates to inhibitors of serine proteases involved in the blood coagulation process such as thrombin (factor IIa), factor Xa, factor VIIa, factor XIIa, kallikrein, plasmin, prolyl endopeptidase and Ig AI Protease.

Thrombin, the last enzyme in the coagulation system, cleaves soluble fibrinogen to fibrin, which is then crosslinked and forms an insoluble gel forming the matrix for a thrombus. When a vessel is damaged, the above process is necessary to stop bleeding. Under normal circumstances there is no measurable amount of thrombin present in plasma. Increase of the thrombin concentration can result in formation of clots, which can lead to thromboembolic disease, one of the most common serious medical problems of our time.

Thrombin contributes to haemostatic control by means of several biological reactions. In addition to its primary function, the conversion of fibrinogen to fibrin, thrombin activates Factor XIII, which is responsible for the crosslinking of fibrin. Thrombin also acts by means of a positive feed back mechanism involving the activation of Factors V and VIII, both of which are necessary for formation of thrombin from prothrombin. Thrombin has another essential role: its binding to platelets initiates platelet release and aggregation which is responsible for primary haemostasis.

The reactions of thrombin are further controlled by natural inhibitors in plasma. The most important of these are antithrombin III and heparin. These two compounds have been isolated and are used therapeutically and prophylactically in conditions where there is an imbalance in the haemostatic mechanism with risk of prothrombin activation.

Orally active synthetic thrombin inhibitors would be useful as alternatives to the parenteral administration of these natural inhibitors. Much research in this area has resulted in the synthesis of good inhibitors of thrombin <u>in vitro</u>, but as yet there is no really good candidate for oral therapeutic use. By imitating amino acid sequences of fibrinogen, the important natural substrate of thrombin, several good short peptide substrates for thrombin have been produced. These peptide substrates have also been derivatised to provide

inhibitors of the enzyme. Thus, the chromogenic substrates D-Phe-Pro-Arg-pNA and D-Phe-Pip-Arg-PNA mimic the sequence preceding the thrombin cleavage site. The corresponding reversible and irreversible inhibitors, D-Phe-Pro-Arginal and D-Phe-Pro-Arg-CH_2Cl show inhibition in vitro in the 10^{-8} M range. Chloromethylketones are generally insufficiently specific to be ideal for therapeutic use, and the peptide aldehyde exemplified above has quite a low LD_{50} value.

Factor Xa is the coagulation enzyme responsible for the generation of thrombin by limited proteolysis of its zymogen, prothrombin. On a weight for weight basis factor Xa is at least 10 times more thrombogenic in vivo than thrombin. This arises from the fact that factor Xa is one step above thrombin in the amplifying cascade system, so that one molecule of factor Xa can generate many molecules of thrombin from its precursor. Its potency may also arise from the relatively slow removal of factor Xa by the body. Thrombin, unlike factor Xa, is rapidly cleared from circulating blood onto high affinity sites on the vessel wall.

The binding of tissue factor to factor VII represents a key event in initiation of blood coagulation after tissue injury. First it catalyses the activation of factor IX and factor X and secondly, trace concentrations of factor Xa and factor IXa (and concievably other enzymes released from cells after tissue injury) convert zymogen factor VII/tissue factor complexes into highly active factor VIIa/tissue factor complexes.

The central position of factor Xa at the junction of the intrinsic and the extrinsic pathways as well as the crucial role of factor VIIa in initiating both the intrinsic and extrinsic coagulation pathways make factors Xa and VIIa suitable targets for modulating the haemostatic process.

Kallikrein is formed from prekallikrein by the action of factor XII, in the presence of a negatively charged surface. Kallikrein in turn can cleave factor XII to factor XIIa, thereby forming a reciprocal activation system. Factor XIIa is the first enzyme of the intrinsic part of the coagulation system. The significance of the contact system is probably as a surface mediated defence mechanism, and a large scale activation of the system is normally seen during shock or disseminated intravascular coagulation (DIC). The role of kallikrein at this stage is to cleave high molecular weight kininogen with the release of the

vasodilator, bradykinin. Bradykinin also causes increased vascular permeability, pain and migration of the neutrophil leucocytes. Inhibitors of kinin formation have been shown to be beneficial in certain types of inflammation, including arthritis and pancreatitis, and may be useful also in the treatment of asthma. The only substance that has attained clinical significance as a kallikrein inhibitor, is aprotinin (Trasylol). Aprotinin is a polypeptide of molecular weight 6,500, and forms a stable complex with proteases, having a binding constant of 10^{-10}-10^{-13} M.

Fibrinolysis is the process of enzymatic dissolution of fibrinogen and fibrin clots. Plasma contains a protein, plasminogen, which under the influence of various activators is converted to plasmin, a proteolytic enzyme, the activity of which resembles that of trypsin. Plasmin breaks down fibrinogen and fibrin to fibrin/fibrinogen degradation products.

Under normal conditions, the fibrinolysis system is in balance with the coagulation system. Small thrombi formed in the blood stream are dissolved enzymatically and the circulation through the vessels is restored by the activation of the fibrinolytic system in the body. If the fibrinolytic activity is too high, it may cause or prolong bleeding. The activity can be inhibited by natural inhibitors in the blood.

Prolyl endopeptidase cleaves peptide bonds on the carboxyl side of proline residues within a peptide chain. It is a serine protease which readily degrades a number of neuropeptides including substance P, neurotensin, thyrotropin-releasing hormone and luteinizing hormone-releasing hormone and which has been associated with the ability of cells to produce interleukin 2 (IL-2). The enzyme is inhibited by benzyloxycarbonyl-prolyl-prolinal with a Ki of 14 nM. Although little is known about the physiological role of prolyl endopeptidase, it may play a prominent role in the regulation of the biological activities of various neuropeptides.

The Ig A proteinase-catalyzed cleavage of Ig A, the predominant form of antibody which comprises the first line of defence against infection, separates the Fc from the antigen-binding Fab regions of the molecule. Such cleavage would be expected to impair or abolish its antimicrobial activity. All Ig A proteinases identified thus for cleave after a proline residue within the hinge region of human Ig A. Peptide prolyl-boronic acids inhibit Ig A 1 proteinases from <u>Neisseria gonorrhoea</u> and <u>Hemophilus influenzae</u> indicating these

enzymes belong to the serine protease family of proteolytic enzymes.

The multiple roles played by thrombin in a variety of physiological processes which have been associated with pathological disorders such as cancer, inflammation and neuronal activity, suggest a potential use of thrombin inhibitors in several indications not strictly related to the cardiovascular system.

Many tumor cells have been shown to elicit procoagulant activity associated with the generation of thrombin. As a consequence, local fibrin deposition and coagulation occur which are thought to be important for tumor growth. Additionally, due to its effects on endothelial cells, thrombin may facilitate the extravasation of tumor cells during metastasis. Hence, thrombin inhibitors may prove beneficial not only in the treatment of certain cancers but also in reducing the hypercoagulability frequently observed in patients during therapy with chemotherapeutic agents.

Thrombin activation of endothelial cells induces a number of pro-inflammatory changes such as synthesis and release of interleukin-1, prostaglandins and platelet-activating factor. Additionally, thrombin induces the exposure of CD62 (alternatively known as GMP-140) and CD63, two adhesion molecules responsible for the adhesion of leukocytes to the endothelial surface. Thrombin also increases vascular permeability of proteins involving neutrophils, and cleaves the interleukin-8-precursor protein, a peptide believed to be involved in respiratory disorders, rheumatoid arthritis and ulcerative colitis.

Thrombin's involvement in all these pro-inflammatory processes make thrombin inhibitors a potential target for use in the therapeutic treatment of inflammation-related pathological disorders.

The activity of the protease nexin-1, a modulator of nerve growth and a specific natural thrombin antagonist, is markedly and specifically decreased in patients with Alzheimer's disease. This, together with the observation that thrombin-like activity was increased in the brains of Alzheimer's patients, suggests that thrombin inhibitors may have potential for limiting or reversing neuronal pathological changes associated with hyperactivity of thrombin or related serine proteases.

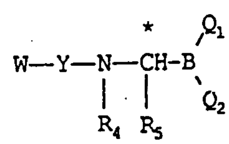
Boronic acids have been studied as inhibitors of various serine esterases and proteases. The first boronic acid-containing amino acid analog to be used as a protease inhibitor was the boronic acid analog of N-acetyl L-phenylalanine, which was used as an inhibitor of chymotrypsin and subtilisin. Peptide boronic acids have been used as inhibitors of chymotrypsin, subtilisin, and elastases.

The interaction of boronic acids with proteases in biological systems is known and various simple boronic acids have been shown to be sufficiently non-toxic for use in humans. Peptide boronic acid inhibitors of elastase have recently been used in animal trials in relation to emphysema. Unlike the peptide chloromethylketones, there was no toxicity reported at biologically effective dosage levels.

European Patent Application 293 881 (Dupont) describes the preparation of peptides comprising C-terminal boronic acid derivatives of lysine, ornithine and arginine, and their use as inhibitors of trypsin-like serine proteases. The other amino acids of the peptides are all either the D- or the L- forms of the 20 naturally-occurring amino acids.

It has now been found that compounds, having superior properties as inhibitors of trypsin-like serine proteases, are obtained when the peptide contains at least one unnatural α-amino acid having a hydrophobic side chain.

Accordingly, the present invention provides compounds of formula I and salts thereof



I

wherein W = H or an N-protecting group;
Y is a sequence of n amino acids selected such that the n+1 amino acid peptide Y-Lys or Y-Arg has an affinity for the active site of a trypsin-like protease, where n is an integer from 1 to 10 preferably 1 to 4, and in which at least one amino acid is an unnatural amino acid having a hydrophobic side chain;
Q_1 and Q_2, which may be the same or different, are selected from -OH, -COR_1, -$CONR_1R_2$, -NR_1R_2, and -OR_3, or Q_1 and Q_2 taken together form a diol residue; R_1, R_2 and R_3, which

may be the same or different, are C_{1-10} alkyl, C_{6-10} aryl, C_{6-10} aralkyl, or phenyl substituted by up to three groups selected from C_{1-4} alkyl, halogen and C_{1-4} alkoxy;

R_4 is hydrogen or C_{1-10}alkyl;

R_5 is a group -A-X

wherein A is $-(CH_2)_z-$ in which z is 2,3,4 or 5; $-CH(CH_3)-(CH_2)_2-$; $-CH_2-CH(CH_3)-CH_2-$; $-(CH_2)_2-CH(CH_3)-$; $-(CH_2)_2-C(CH_3)_2-$; $-CH(CH_3)-(CH_2)_3-$; $-CH_2-CH(CH_3)-(CH_2)_2-$; $-CH_2-CH_2-CH(CH_3)-CH_2$; $-(CH_2)_2C(CH_3)_2-CH_2-$; $(CH_2)_3-CH(CH_3)-$; $-(CH_2)_3-C(CH_3)_2$: $-(CH_2)_3CH(CH_3)CH_2-$; C_{6-10}aryl, C_{6-10}aralkyl

and wherein X is OH, SH, NR_6R_7 or phenyl,

wherein R_6 is H or C_{1-10}alkyl, and R_7 is C_{1-10}alkyl, $-CO-R_8$, $CS-R_8$, or SO_2-R_8

wherein R_8 is H or C_{1-10}alkyl, C_{1-10}alkoxy, C_{6-10}aryl, C_{6-10}aralkyl optionally substituted by up to three groups selected from halogen, OH, C_{1-4}alkoxy, $C(NH)R_9$, $NR_{10}R_{11}$ and $C(O)OR_6$ (wherein R_6 is as defined above),

wherein R_9 is C_{1-3}alkyl or $N(CH_3)_2$ and wherein R_{10} and R_{11} (which may be the same or different) are H or C_{1-10}alkyl,

and the asymmetric carbon atom marked * may have the D- or L-configuration, and the compounds may comprise any mixture of these.

By an unnatural amino acid is meant any amino acid other than D- or L- Ala, Arg, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

Preferably the N-protecting group W is of formula $H(CH_2CH_2O)_p-$ where p = 3-30; $R_{12}CO-$; $R_{13}OCO-$ or $R_{14}SO_2-$

in which R_{12} is C_{1-6} alkyl or $H(CH_2-O-CH_2)_p$ wherein p is as defined above; R_{13} is C_{1-6} alkyl, phenyl, benzyl or naphthyl; and R_{14} is phenyl, naphthyl or C_{1-4} alkylphenyl;

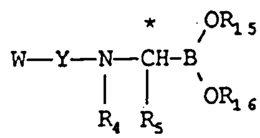
of which $R_{13}OCO-$ is particularly preferred. The most preferred protecting groups are those of formula $R_{13}'OCO-$ in which R_{13}' is <u>tert</u>- butyl (designated Boc), and in which R_{13}' is benzyl (designated Z).

Preferably R_5 is R_5' where R_5' is $-(CH_2)_{z'}-X'$, in which X' is OH, phenyl, NH-CO-R_8', NH-CS-R_8', $NHSO_2R_8'$ or $NR_{10}'R_{11}'$,

wherein R_8' is H, or C_{1-3}alkyl or C_{1-3}alkoxy and wherein R_{10}' and R_{11}' which may be the same or different are H or C_{1-3}alkyl optionally substituted with up to 3 halogen atoms, and z' is 2,3 or 4.

More preferably R_5 is R_5'' where R_5'' is $-(CH_2)_{z'}-X''$, in which X'' is OH, C_6H_5, C_6H_4Cl, $C_6H_4N(Me)_2$, NH-CO-CH_3, NH-CHO, NH-CS-$NHCH_3$, NH-CO-$CH(CH_3)_2$, NH-CO-CH_2-CH_3, NH-CHS, NH-CO-CH_2Cl, NH-CO-OCH_3 and z' is as defined above.

Preferred compounds are those of formula Ia

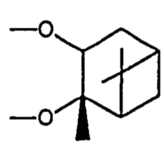


Ia

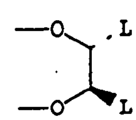
wherein: W, Y, R_4 and R_5 are as defined above and R_{15} and R_{16} represent the residue of a dihydroxy compound.

Useful examples of dihydroxy compounds are, 2,3-butanediol; catechol: 2,3-dimethyl; 2',3'-butandiol; cyclohexanediol; ethylene glycol; 1,2-hexanediol; 2,3-hexanediol; diethanolamine or aliphatic or aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon atoms.

Particularly preferred are those compounds in which Q_1 and Q_2 taken together represent the group OPin of formula a) or the group of formula b)

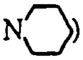


a)



b)

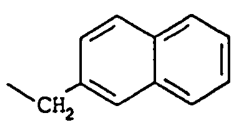
in which L is a C_{1-4}alkyl group, especially methyl, i-Pr, n-Pr or n-Bu.

The amino acids constituting Y are α-amino acids which may be selected from the L-amino acids naturally occurring in proteins, their corresponding enantiomeric D-amino acids or chemically modified alpha-amino acids such as glutamic acid gamma-piperidide (Glu N) or pipecolic acid (Pip), provided that at least one amino acid is an unnatural amino acid having a hydrophobic side chain.

The unnatural amino acid may be an alkylated natural amino acid such as O-alkyl or S-alkyl-cysteine. However, preferred unnatural amino acids are of formula II

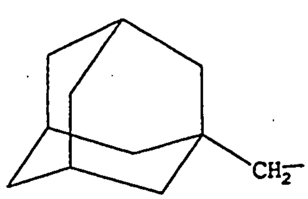
$$-\mathrm{NH}-\underset{\displaystyle |}{\overset{\displaystyle R_{17}}{\overset{|}{\mathrm{C}}}}\!\mathrm{H}-\underset{\underset{\displaystyle \mathrm{O}}{\|}}{\mathrm{C}}- \qquad \text{(II)}$$

in which R_{17} is a hydrophobic group. Preferred hydrophobic groups consist of a methylene group linked to an aromatic group optionally substituted by a polar group or an alicyclic group having at least two rings and no polar substituents, or to a tert. butyl or trimethylsilyl group. Preferably R_{17} is R_{17}' where R_{17}' is a group of formulae c), d), e), f), g), h) or i)

c) 

$-CH_2-Si(CH_3)_3$

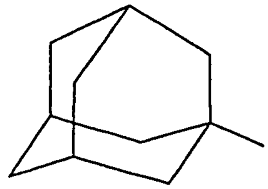
d)



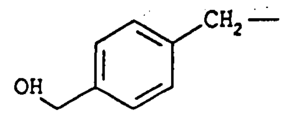
e)

$-CH_2-C(CH_3)_3$

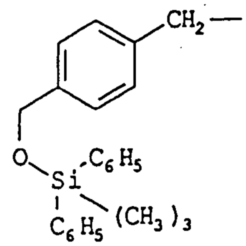
f)



g)



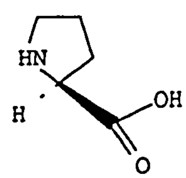
h)



i)

The unnatural amino acids of formula II may be in D- or L- form or any mixture of these, but are preferably in D-form.

More preferred compounds are thrombin inhibitors of formula I in which Y is a sequence of two amino acids, of which the N-terminal amino acid is the unnatural amino acid and the other amino acid is L-proline (Pro), of formula

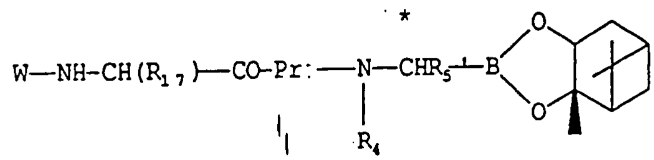


These more preferred compounds have the formula I'

$$\mathrm{W - NH - CH(R_{17}) - CO - Pro - \underset{\displaystyle R_4}{\underset{|}{N}} - \underset{\displaystyle R_5}{\underset{|}{\overset{*}{C}H}} - B} \begin{matrix} \diagup Q_1 \\ \diagdown Q_2 \end{matrix} \qquad \mathrm{I'}$$

in which W, R_4, R_5, R_{17}, Q_1, and Q_2 are as defined above.

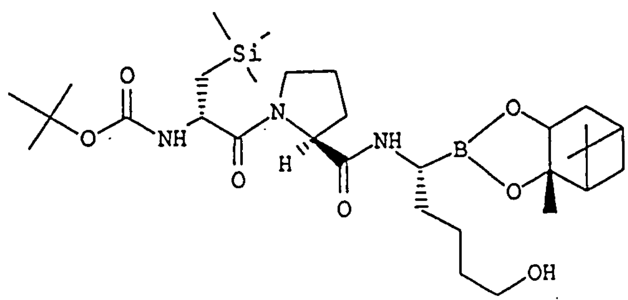
Particularly preferred compounds are those of formula I"



I''

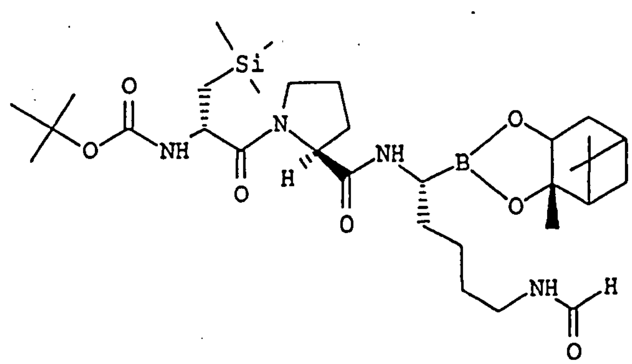
in which W, R_4, R_5' and R_{17} are as defined above.

The most preferred compounds are the compound of formula III,



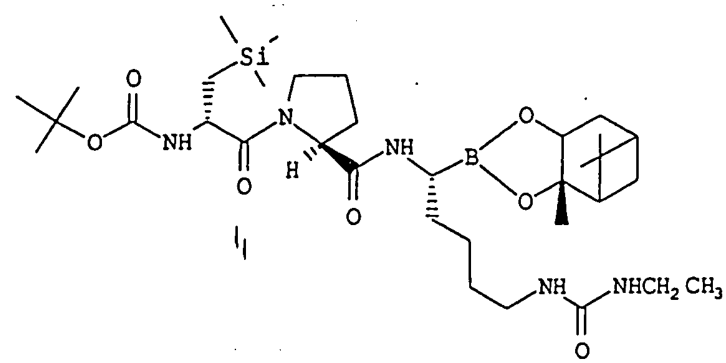
III

the compound of formula IV,



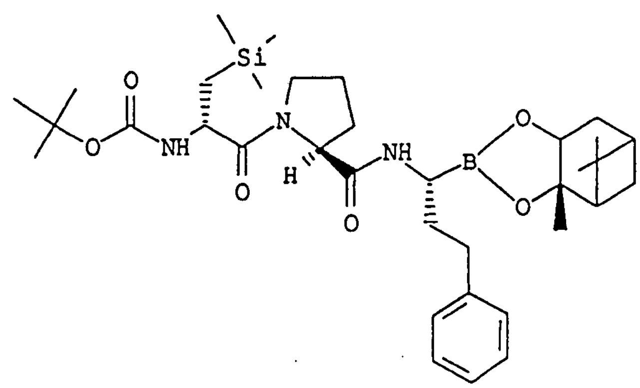
IV

the compound of formula V



V

and the compound of formula VI



VI

A peptide is considered to have an affinity for the active site of trypsin-like proteases if the dissociation constant of the peptide-protease complex has a value of 10^{-5} M or lower.

The compounds of formula I in which X is NHCHO or NHCOalkyl may be prepared by reacting a compound of formula I in which X is $-NH_2$ with an active form of the corresponding acid.

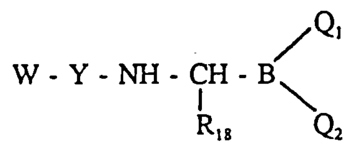
The compounds of formula I in which X is NHCOalkoxy may be prepared by treating a compound of formula I in which X is $-NH_2$ with N,O-Bis (trimethylsilyl) acetamide followed by addition of an active form of the corresponding ester.

The compounds of formula I in which X is NHC(S)NHalkyl may be prepared by

reacting a compound of formula I in which X is $-NH_2$ with the corresponding alkyl isothiocyanate in an organic solvent.

Compounds of formula I in which X is $-NH_2$ may in turn be produced by hydrogenation of a compound of formula I in which X is $-N_3$. Hydrogenation may be carried out under standard conditions using for example a Pd/C catalyst.

Compounds of formula I in which X is $-N_3$ may be produced by reaction of a compound of formula VI

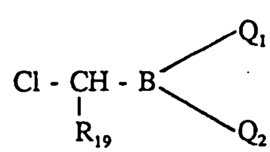


VI

in which W, Y, Q_1 and Q_2 are defined above and R_{18} is -A-Br wherein A is as defined above with sodium azide in a polar aprotic solvent such as dimethyl sulphoxide.

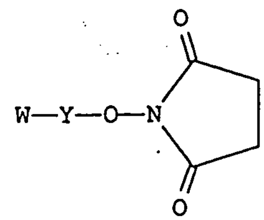
The compounds of formula I in which X is -NHCHS may be prepared by reacting the compound of formula I in which X is NHCHO with Laweson reagent in an organic solvent such as toluol.

The compounds of formula I in which X is optionally substituted phenyl may be obtained by the reaction of a compound of formula VII



VII

in which Q_1 and Q_2 are defined above and R_{19} is -A-phenyl (optionally substituted) wherein A is as defined above, with $LiN[Si(CH_3)_3]_2$ followed by hydrolysis with an excess of acid and coupling with a protected peptide of formula VIII



VIII

wherein W and Y are as defined above

The reaction is preferably carried out in a dry aprotic polar solvent, for example tetrahydrofuran, at a temperature between -78°C and room temperature.

The intermediates of formula VII may be obtained by the method of Matteson et al, Organometallics 3 1284-8 (1984).

The protected peptide of formula VIII may be prepared by methods which are conventional in peptide chemistry, starting from the desired unnatural amino acid. Such amino acids are either commercially available (for example the amino acid in which R_{16} is the group c) or may be prepared by methods analogous to those described in the literature, e.g. Angew. Chem. 93, 793 (1981) and J. Am. Chem. Soc. 109, 6881 (1987).

The compounds of fomula I in which X is OH may be obtained by reacting a compound of formula I in which X is $-OSi(CH_3)_2\ C(CH_3)_3$ with a desilylating agent, such as tetrabutylammonium-fluoride.

The compounds of formula I are useful as inhibitors of trypsin-like proteases and may be used in vitro for diagnostic and mechanistic studies of these enzymes. Furthermore, because of their inhibitory action they are indicated for use in the prevention or treatment of diseases caused by an excess of an enzyme in a regulatory system, for example control of coagulation and fibrinolysis.

The compounds of the invention have trypsin-like serine protease inhibiting properties, as indicated in the following test methods:

a) Enzyme inhibition kinetics

Test substance is dissolved in cremophor/ethanol (1:1) or DMSO and diluted with distilled water to yield a 1 mM stock solution. Further dilutions are made into the assay buffer (100 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl and 0.1 % bovine serum albumin). Kinetic assay are performed using a 96-microwell plate; each well contains 50 µl substrate, 100 µl test compound and 100 µl enzyme in buffer. Final concentrations of substrate and enzyme are as follows: 160 pM α-thrombin and 100 µl Pefachrome TH (K_m = 6.9 µM), 800 pM human plasmin and 200 µM Pefachrome PL (K_m = 66.4 µM), and 260 pM bovine pancreatic trypsin and 500 µM Pefachrome TRY (K_m = 167.7 µM). Assays are initiated by adding enzyme to solutions containing the substance to be tested and substrate. The release of p-nitroaniline from the hydrolysis of the substrate is followed for 30 min by measuring the increase in optical density at 405 nm with a Thermomax microwell kinetic reader (Molecular Devices, Menlo Park CA, USA). When the inhibited steady-state rate is achieved rapidly, the inhibition constant (K_i) is determined by fitting the data by weighted linear regression to the Dixon equation (Biochem. J. 1953, 55:170-171). For slow-, tight-binding inhibitors, the mechanism of inhibition may be described by the following scheme:

$$\begin{array}{ccccc} E + S & \overset{K_m}{\rightleftharpoons} & ES & \xrightarrow{k_{net}} & E + P \\ + & & & & \\ I & & & & \\ k_{on} \downarrow\uparrow k_{off} & & & & K_i = k_{off}/k_{on} \\ EI & & & & \end{array}$$

where E, S, P, and I are the enzyme, substrate, product (p-nitroaniline) and inhibiting substance to be tested, respectively, and k_{on} and k_{off} are the association and dissociaton rate constants for the inhibition (Tapparelli et al., J. Biol. Chem., 268 (1993), 4734-4741). Progress-curve data for the formation of p-nitroaniline in the presence of different concentrations of inhibitor are fitted by nonlinear regression to the equation for the mechanism presented in the scheme (Morrison and Walsh, Adv. Enzymol. Relat. Areas

Mol. Biol. 1988, 61:201-301). These analyses yield estimates for the apparent values of k_{on}, k_{off} and K_i which are corrected for the presence of substrate as described by Morrison and Walsh to give the true values. Under the described experimental conditions a K_i of 13 nM is observed for the compound of Example 1.

b) In vitro coagulation assays

In vitro coagulation assays are performed with pooled citrated human plasma. Substance to be tested or solvent are incubated with plasma for 10 min at 37°C prior to assay. Thrombin time(TT) determinations are performed at a final thrombin concentration of 5 U/ml. Activated partial thromboplastin time (APTT) is determined by incubating 0.1 ml plasma ± substance to be tested with 0.1 ml purified soya bean phospholipid in ellagic acid (Actin-FS) for 4 min at 37°C and followed by the addition of 0.1 ml $CaCl_2$ (50mM). In this test method, compounds of the invention significantly increase TT and APTT at a concentration of from 0.1 µM to 0.5 µM. With the compound of Example 1, prolongation of TT above 300 sec is achieved at 1.8 µM, elevation of APTT to 2x the control value is achieved at 4.4 µM.

c) In vitro platelet aggregation assay

Washed human platelets are prepared by a modification of the method of Ardlie (Br. J. Pharmacol. 1970, 19:7-17). Washed platelet suspension (0.46 ml) is kept at 37°C and stirred at 1100 rpm. After the addition of the substance to be tested or solvent, the platelets are maintained for 2 min before aggregation is induced by 1 nM human thrombin. The extent of platelet aggregation is quantitated by the maximum aggregation amplitude and inhibition of aggregation rate observed in the absence of inhibitor. In this test method, compounds of the invention significantly inhibit platelet aggregation at a low nM concentration range. The compound of Example 1 has an IC_{50} value of 3.8 nM.

Those compounds of the invention which are thrombin or factor Xa inhibitors have anti-thrombogenic properties and may be employed when an anti-thrombogenic agent is needed. Generally, these compounds may be administered orally or parenterally to a host to obtain an anti-thrombogenic effect. In the case of larger mammals such as humans, the

compounds may be administered alone or in combination with pharmaceutical carriers or diluents at a dose of from 0.02 to 15 mg/Kg of body weight and preferably 1-10 mg/Kg to obtain the anti-thrombogenic effect, and may be given as a single dose or in multiple doses or as a sustained release formulation. When an extracorporeal blood loop is to be established for a patient, 0.1-1 mg/Kg may be administered intravenously. For use with whole blood from 1-10 mg per liter may be provided to prevent coagulation. Pharmaceutical diluents are well known and include sugars, starches and water which may be used to make tablets, capsules, injectable solutions and the like. The compounds of the invention may be added to blood for the purpose of preventing coagulation of the blood in blood collection or distribution containers, tubing or implantable devices which come into contact with blood.

In the arterio-venous shunt thrombosis model in the rat, a modification of the model described by Butler et al. (Blood Coag. Fibrinol., Vol. 3, (1992), page 155), the compounds of the invention prevent thrombus formation in a dose dependent manner. Using the compound of Example 1, thrombus formation is inhibited by 21% at a dose of 0.3 mg/kg i.v. and blocked by a dose of 3 mg/kg i.v.

Also, in view of their trypsin-like serine protease inhibition activity, the compounds of formula I are indicated for use for inhibiting vascular remodelling (proliferation, migration) following venous or arterial surgery or other forms of vascular damage.

Percutaneous transluminal coronary angioplasty (PTCA) has become a common surgical procedure for the treatment of atherosclerotic plaques occluding coronary arteries. Angioplasty is also performed, albeit to a lesser extent, on peripheral vessels such as the femoral and renal arteries. The splitting/compression of an atherosclerotic plaque and stretching of the arterial wall during balloon catheter angioplasty additionally results in injury to (or removal of) the endothelial cells and possibly also damage to the underlying smooth muscle cells. This accounts for the primary complications of PTCA, acute reocclusion (build up of an occlusive platelet thrombus on the exposed thrombogenic vessel surface) and chronic restenosis (primarily due to proliferation and migration of smooth muscle cells). Acute restenosis, occuring in ~5% of patients within 24 hours after PTCA, is life threatening and must be surgically dealt with immediately. Chronic restenosis, diagnosed 3-6 months post-surgery in around 30% of patients, leads to chest pain but is

only life threatening if the occlusion is 100% (giving rise to myocardial infarction if the plaque should rupture).

The possible involvement of thrombin in the vascular response to injury has recently come under scrutiny. By its action on platelets, thrombin could be involved in the early platelet recruitement and activation at the site of damage, and by virtue of its mitogenic action on smooth muscle cells, thrombin could also contribute to intimal thickening. Thrombin concentrations are likely to be elevated at sites of vascular injury because of continued activation of the coagulation pathways in the absence of a normal endothelial layer and also due to release of clot-bound thrombin during the thrombolytic process. Additionally, thrombin becomes bound to the extracellular matrix where it can remain active, and protected from the inhibitory effect of circulating antithrombins, for prolonged periods.

The interaction of platelets with a severly injured vessel wall appears in part to be dependent on local thrombin generation.

Whilst the accumulated evidence points to thrombin inhibition being a potentially attractive principle to limit restenosis following balloon angioplasty, available agents all have the disadvantage of needing to be administered parenterally (or locally) in order to obtain sufficient local concentrations to demonstrate an effect, or other side effects may be limiting.

The compounds of the invention may be used to inhibit vascular remodelling at doses similar to those at which they are used as anti-thrombogenic agents.

Advantageously the compounds of the invention are orally active, have rapid onset of activity and low toxicity. In addition, these compounds may have particular utility in the treatment of individuals who are hypersensitive to compounds such as heparin.

In the following examples, the symbols have the following meanings:

Z	= benzyloxycarbonyl
Boc	= t-butyloxycarbonyl
Ac	= acetyl
MeOH	= methyl alcohol
EtOAc	= ethyl acetate
DMF	= dimethyl formamide
DCC	= dicyclohexylcarbodiimide
HONSu	= N-hydroxy-succinimide
OPin	= pinanediol
THF	= tetrahydrofuran
n-Bu	= n-butyl
Np	= p-nitrophenyl
TLC	= thin layer chromatography
Bzl	= benzyl
TMSal	= trimethylsilylalanine
BoroPro-0Pin	= analog of proline in which the -C00H group is replaced by B-0Pin
BoroLys	= $-NH-CH-(CH_2-CH_2-CH_2-CH_2-NH_2)B-$

Example 1

Boc-D-TMSal-Pro-NH-CH[(CH_2)$_4$OH]B-OPin

A. Boc-D-TMSal-OH

21.5 g (113.7 mmol) D-TMSal ethyl ester, prepared according to the procedure given in Angew. Chem. 93, 793 (1981), is dissolved in CH_2Cl_2 and a solution of an excess of Boc$_2$O in CH_2Cl_2 is added. After 15 h at room temperature, 500 ml of ice-cold 0.25 N hydrochloric acid is added. The organic layer is washed with 5 % $NaHCO_3$ and brine, then dried over Na_2SO_4 and concentrated in vacuo.

The crude material (colourless oil) is used directly in the saponification step. It is dissolved in methanol, cooled to 0°C, mixed with 510 ml of 1 N NaOH and stirred at 0°C for 3 h. After acidification to pH 1 with 1 N HCl the mixture is extracted several times with ether. The organic layers are combined, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The product (29.7 g oil) is used in the next step without further purification.

B. Boc-D-TMSal-Pro-ONSu

29.7 g (113.7 mmol) Boc-D-TMSal-OH and 19.0 g (136.3 mmol) p-nitrophenol are dissolved in EtOAc. After cooling to 0°C, 23.4 g (113.6 mmol) DCC is added and the mixture is stirred for 1 h at 0°C and then for 15 h at room temperature. The precipitate is filtered off and washed with EtOAc and the filtrate is concentrated in vacuo. The resulting oil is purified by flash chromatography (9:1, hexane/EtOAc) to give the desired Boc-D-TMSal-ONp as white crystals.

51.6 g (113.7 mmol) Boc-D-TMSal-ONp is dissolved in THF and an aqueous solution of equimolar amounts of proline and Et_3N is added. After 20 h at room temperature, the THF is removed in vacuo and the aqueous residue is diluted with water and then extracted several times with EtOAc. The pH of the aqueous layer is adjusted to 3 by adding 10 % citric acid. The resulting oily product is extracted several times with EtOAc. The combined organic layers are washed with brine, dried over Na_2SO_4 and

concentrated in vacuo. The colourless oil is recrystallized from ether/hexane to give the dipeptide Boc-D-TMSal-Pro-OH as a white crystalline compound, mp: 176°C.

26.0 g (72.5 mmol) of the resulting dipeptide is dissolved in EtOAc. After cooling to 0°C, 9.8 g (85.5 mmol) HONSu and 14.9 g (72.3 mmol) DCC are added. The mixture is stirred for 3 h at 0°C and then for an additional 15 h at room temperature. The mixture is recooled to 0°C, the dicyclohexylurea is filtered off and washed several times with EtOAc. The filtrate is washed with aqueous 0.1 M Na_2CO_3 and then with aqueous 2 % $KHSO_4$. After drying over Na_2SO_4 and concentration in vacuo, Boc-D-TMSal-Pro-ONSu is obtained as a white foam.

C. (+)-Pinanediol-(*S*)-1-chloro-5-*t*-butyldimethylsiloxy-pentane-1-boronate

10.5 ml (120 mmol) 3-Butene-1-ol, 22.3 g (144 mmol) *t*-butyldimethylsilylchloride and 20.4 g (300 mmol) imidazole are dissolved in 60 ml DMF and stirred overnight at 35°C. The two layers are separated and the product layer is washed with 2 N tartaric acid, water and brine. After drying over Na_2SO_4 and concentration in vacuo, 1-*t*-butyldimethylsiloxy-3-butene is obtained as colourless oil.

7.45 g (40 mmol) of this product is stirred for 24 h with 5.1 g (40 mmol) catecholborane at 125°C, yielding a yellow-brown oil. This oil is added to 6.9 g (40 mmol) (+)-pinanediol in 30 ml THF and stirred overnight. After flash chromatography (95:9, hexane/EtOAc) (+)-pinanediol-4-*t*-butyldimethylsiloxy-butane-1-boronate is obtained.

Over a 20 min period 17 ml (27.0 mmol) BuLi (1.6 M in hexane) is added to a precooled (-100°C) solution of 2.8 ml CH_2Cl_2 and 49 ml THF. After stirring for 15 min at 100°C, 9.0 g (24.5 mmol) of the pinanediolboronate product obtained above in 24 ml THF is added over a 20 min period. The reaction mixture is again stirred for 15 min at -100°C, after which 1.24 g (12.3 mmol) $ZnCl_2$ (solid) is added. The reaction mixture is allowed to warm up to room temperature overnight. After concentration in vacuo the residue is dissolved in ether/H_2O. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. (+)-pinanediol-(*S*)-1-chloro-5-*t*-butyldimethylsiloxy-butane-1-

boronate is obtained as yellow-orange oil.

D. Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$OSi(t-Bu)Me_2]B-OPin

12.8 ml (20.4 mmol) Butyllithium (1.6 M in hexane) is added to a precooled (-78°C) solution of 4.3 ml (20.4 mmol) hexamethyldisilazane in 30 ml THF. The reaction mixture is stirred for 1 h at room temperature, then cooled down to -78°C again. 8.5 g (20.4 mmol) (+)-pinanediol-(*S*)-1-chloro-5-*t*-butyldimethylsiloxy-butane-1-boronate in THF is added. After stirring for 1 h at -78°C, the solution is allowed to warm overnight to room temperature. After recooling to -78°C 3 mol equivalents of HCl in dioxane are added. The mixture is stirred for 1 h at -78°C, then for 2 h at room temperature. After cooling down to -20°C, a solution of 9.28 g (40.8 mmol) of the active ester of Example 1, step B in CH_2Cl_2 is added, followed by the addition of 5.67 ml (40.8 mmol) triethylamine. The mixture is stirred for 1 h at -20°C and for 2 h at room temperature, then filtered. After concentration in vacuo, the residue is dissolved in ether/H_2O. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. After flash chromatography (6:4, hexane/EtOAc), the desired product is obtained; $[\alpha]_D^{20}$ = - 45.6° (c = 0.5 in MeOH); MH^+ = 736.

E. Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$OH]B-OPin

600 mg (0.816 mmol) of the siloxy compound obtained in step D of Example 1 is dissolved in THF and 514 mg (1.62 mmol) tetrabutylammonium fluoride is added. After stirring overnight at ambient temperature, the mixture is concentrated in vacuo and dissolved in ether/H_2O. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. The side product is removed with EtOAc on silicagel, then the product is eluated with EtOAc/EtOH (9:1), yielding the title product as a white foam; $[\alpha]_D^{20}$ = - 72.4° (c = 0.5 in MeOH); MH^+ = 622.

Example 2-4

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHCHO]B-OPin

A) (+) Pinanediol-(*S*)-1-chloro-5-bromo-pentane-1-boronate

20.8 ml (203.3 mmol) 4-Bromo-1-butene is reacted with 924.4 g (203.3 mmol) catecholborane at 100°C over 16 h. The crude product is distilled in vacuo to give 4-bromo-butane-1-boronate as a white crystalline product.

27.7 g (163 mmol) (+)-Pinandiol is dissolved in THF and 41.6 g (163 mmol) of 4-bromo-butane-1-boronate as synthesized above is added. After 1 h at room temperatur, the THF is removed in vacuo and the residue is purified by flash chromatography (9:1, hexane/EtOAc) to give (+)-pinanediol-4-bromo-butane-1-boronate as a colourless oil.

The desired (+)-pinanediol-(S)-1-chloro-5-bromo-pentane-1-boronate is prepared according to the procedure given in Organometallics 3, 1284 (1984). 9.8 ml CH_2Cl_2 in THF is cooled to -100°C and 71.6 ml (114.5 mmol) n-butyllithium (1.6 M in hexane) is added over 20 min. After 15 min at -100°C, a cold (-78°C) solution of 32.8 g (104.1 mmol) (+)-pinanediol-5-bromo-pentane-1-boronate in THF is added. After an additional 1 h at -100°C, 7.1 g (52.0 mmol) anhydrous $ZnCl_2$ in THF is added. After an additional 15 min at -100°C, the reaction mixture is warmed to room temperature. The solvent is removed in vacuo, the residue diluted with hexane/water and extracted several times with hexane. After drying over Na_2SO_4 and removal of the solvent in vacuo, (+)-pinanediol-(*S*)-1-chloro-5-bromo-pentane-1-boronate is obtained as a yellow oil which is used directly in the next step without further purification.

B) Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$Br]B-OPin

A solution of 65.2 ml (66.2 mmol) $LiN(SiMe_3)_2$ (1 M in THF) in THF is cooled to -78°C. 23.7 g (65.2 mmol) α-Chloro-boronate of Example 2, step A in THF is added. After stirring for 1 h at -78°C, the mixture is stirred for 15 h at room temperature.

After this period, the reaction mixture is recooled to -78°C. 29.8 ml (6.56 N solution, 196 mmol) HCl in dioxane is added and the solution stirred for 45 min at -78°C and then for 2 h at room temperature. The mixture is cooled to -15°C, 29.7 g (65.2 mmol) Boc-TMSal-Pro-ONSu of Example 1, step B in CH_2Cl_2 is added, followed

by 18.1 ml (130.4 mmol) triethylamine to start the coupling reaction. After stirring at -15°C for 1 h, the mixture is stirred for 2 h at room temperature. The mixture is then filtered over Hyflo and concentrated in vacuo. The residue is diluted with ether/water and extracted several times with ether. After drying over Na_2SO_4 and concentration in vacuo, the desired Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$Br]B-OPin is obtained as white crystals from ether/hexane, mp: 74°C.

C) Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4N_3$]B-OPin

33.3 g (48.6 mmol) of the product of Example 2, step B is dissolved in DMSO and 6.3 g (97.2 mmol) sodium azide is added. The mixture is stirred for 6 h at room temperature. Ether/ice water is added, and the resulting oil is crystallized to give Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4N_3$]B-OPin as a white crystalline compound, mp: 69-70°C; $[\alpha]_D^{20}$ = - 56.6° (c = 1.0 in MeOH); MH^+ = 647.

D) Boc-D-TMSal-Pro-BoroLys-OPin

22.0 g (34.0 mmol) of the azide of Example 2, step C is dissolved in EtOAc and hydrogenated in the presence of 4.0 g of 10% Pd/C. After 9 h, the catalyst is removed and the solution is concentrated in vacuo. The resulting foam is dissolved in EtOAc and crystallized to give the desired Boc-TMSal-Pro-BoroLys-OPin as white crystals. mp: 128-129°C; $[\alpha]_D^{20}$ = - 40.8° (c = 0.5 in CH_2Cl_2); MH^+ = 621.

E) Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHCHO]B-OPin

0.224 ml (5.95 mmol) Formic acid and 0.530 g (5.2 mmol) acetic anhydride are stirred 2 h at 60°C. The mixture is cooled to 0°C and 1.86 g (3.00 mmol) of the borolysine obtained in step D of Example 2 dissolved in THF is added. After 10 min stirring at 0°C, the reaction mixture is stirred at room temperature overnight. 80 ml of icewater is added, the solution extracted several times with ether and washed with brine. After drying over Na_2SO_4 and concentration in vacuo, the desired product is obtained as a white foam which can be crystallized from ether/hexane; mp: 93-99°C; $[\alpha]_D^{20}$ = - 77.8° (c = 0.5 in MeOH); MH^+ = 649.

The Boc-D-TMSal-Pro-NH-CH[$(CH_2)_5$NHCHO]B-OPin ($[\alpha]_D^{20}$ = - 47.2° (c = 0.5 in MeOH); MH^+ = 663) and Boc-D-TMSal-Pro-NH-CH[$(CH_2)_3$NHCHO]B-OPin ($[\alpha]_D^{20}$ = - 68.0° (c = 0.5 in MeOH); MH^+ = 635) products are obtained analogously.

Example 5-14

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)CH_3]B-OPin

20 µl (0.25 mmol) Pyridine and 18 µl (0.25 mmol) acetyl chloride in CH_2Cl_2 are cooled to 0°C, 155.2 mg (0.25 mmol) of the borolysine product of Example 2, step D is added and the solution is stirred for 1 h. Water is added, the product extracted several times with ether, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The desired product is obtained as an oil; $[\alpha]_D^{20}$ = - 62.6° (c = 0.5 in MeOH); MH^+ = 663.

The following (Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)R]B-OPin) products wherein R is as indicated in the table below are prepared analogously from the corresponding acyl chlorides or bromides:

Ex.	R	$[\alpha]_D^{20}$ (c = 0.5 in MeOH)	MH^+
6	Et	- 55.8°	667
7	i-Pr	- 60.2°	691
8	CH_2F	- 58.8°	681
9	CH_2Br	- 58.8°	741
10	CH_2OMe	- 79.8°	693
11	CO_2Me	- 64.8°	707
12	CO_2Et	- 59.6 °	721
13	NMe_2	- 64 2°	692
14	OCH_2Cl	- 62.0°	713

Example 15-16

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)CH_2Cl]B-OPin

124.2 mg (0.2 mmol) of the borolysine product of Example 2, step D in THF is cooled to 0°C and 35.2 mg (0.2 mmol) monochloroacetic anhydrid is added. After stirring for 3 h at room temperature, the solvent is removed in vacuo and the product is obtained as a white foam; $[\alpha]_D^{20}$ = - 57.8° (c = 0.5 in MeOH); MH^+ = 697.

The Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)CCl_3]B-OPin product is prepared analogously; $[\alpha]_D^{20}$ = - 54.0° (c = 0.5 in MeOH); MH^+ = 765.

Example 17

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)OCH_3]B-OPin

58.7 µl (0.24 mmol) N,O-Bis(trimethylsilyl)acetamide in CH_2Cl_2 is added to 124.2 mg (0.20 mmol) of the borolysine product of Example 2, step D in CH_2Cl_2 and stirred for 1 h at room temperature. After cooling to 0°C, 19.5 µl (0.24 mmol) methyl chloroformate in CH_2Cl_2 is added and the mixture stirred for 5 h at 0°C. The CH_2Cl_2 is removed and pH 7 buffer is added at 0°C. The product is extracted several times with ether, washed with brine, dried over Na_2SO_4 and concentrated in vacuo, yielding a white foam; $[\alpha]_D^{20}$ = - 59.6° (c = 0.5 in MeOH); MH^+ = 679.

Example 18-22

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(S)NHCH_3]B-OPin

124.2 mg (0.2 mmol) of the borolysine product of example 2, step D in CH_2Cl_2 is cooled to 0°C and 15 mg (0.2 mmol) methyl isothiocyanate is added. After stirring 7 h at room temperature, the solvent is removed in vacuo and the product is obtained as a white foam, which can be crystallized from ether/hexane; mp: 109-113°C; $[\alpha]_D^{20}$ = - 66.8° (c = 0.5 in MeOH); MH^+ = 694.

Analogously Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(S)NHEt]B-OPin ($[\alpha]_D^{20}$ = - 66.4° (c = 0.5 in MeOH); MH^+ = 708), Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)NHCH_3]B-OPin ($[\alpha]_D^{20}$ = - 62.2° (c = 0.5 in MeOH); MH^+ = 678), Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)-NHEt]B-OPin ($[\alpha]_D^{20}$ = - 65.6° (c = 0.5 in MeOH); MH^+ = 692) and Boc-D-TMSal-Pro-NH-CH[$(CH_2)_3$NHC(O)NHEt]B-OPin ($[\alpha]_D^{20}$ = - 71.0° (c = 0.5 in MeOH); MH^+ = 678), are obtained from the corresponding iso(thio)cyanates.

Example 23

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHCHS]B-OPin

217 mg (0.35 mmol) of the formamide product of Example 2, step E in toluene is treated with 70.8 mg (0.175 mmol) Laweson-Reagent. After stirring for 1.5 h at room temperature, toluene is removed in vacuo. The desired product is crystallized from ether; mp: 123-125°C; $[\alpha]_D^{20}$ = - 74.8° (c = 0.5 in MeOH); MH^+ = 665.

Example 24

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(S)CH_3]B-OPin

124 mg (0.2 mmol) of the borolysine product of Example 2, step D is dissolved in MeOH and 23 µl (0.2 mmol) ethyl dithioacetate in MeOH is added at room temperature. After stirring for 2.5 h at 60°C, the solvent is removed in vacuo. The residue is purified by RP-chromatography (7:3, EtOH/H_2O) and the desired product is obtained as a white foam; $[\alpha]_D^{20}$ = - 65.8° (c = 0.5 in MeOH); MH^+ = 679.

Example 25-26

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)NH_2]B-OPin

248.3 mg (0.4 mmol) of the borolysine product of Example 2, step D and 0.4 ml 1 N HCl in H_2O is warmed to 50°C. After 5 min, 33.2 mg (0.4 mmol) of potassium cyanate is added in small portions. After 6 h at 50°C water is added, the product

extracted with EtOAc, dried over Na_2SO_4 and concentrated in vacuo. After flash chromatography (1:9, hexane/EtOAc), the desired product is obtained; $[\alpha]_D^{20}$ = - 71.7° (c = 0.5 in MeOH); MH^+ = 664.

The Boc-D-TMSal-Pro-NH-CH[$(CH_2)_3$NHC(O)NH_2]B-OPin product is prepared analogously; $[\alpha]_D^{20}$ = - 54.0° (c = 0.5 in MeOH); MH^+ = 765.

Example 27

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)CH_2OH]B-OPin

48 µl (0.6 mmol) pyridine and 99.6 µl (0.6 mmol) benzyloxyacetyl chloride in THF are cooled to 0°C and 372.4 mg (0.6 mmol) of the borolysine product of Example 2, step D is added. After 3 h at 0°C, water is added, the product extracted several times with ether, dried over Na_2SO_4 and concentrated in vacuo.

The resulting white foam (370 mg, 0.48 mmol) is dissolved in EtOH and hydrogenated in the presence of 0.5 g of 10% Pd/C at 40 psi. After 12 h, the catalyst is removed and the solution is concentrated in vacuo. After flash chromatography (9:1, EtOAc/EtOH), the desired product is obtained; $[\alpha]_D^{20}$ = - 67.0° (c = 0.5 in MeOH); MH^+ = 679.

Example 28

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHSO_2CH_3]B-OPin

19.8 µl (0.25 mmol) methane sulfonic acid chloride in THF at 0°C is added to 20 µl (0.25 mmol) pyridine. 155.2 mg (0.25 mmol) of the borolysine product of Example 2, step D in THF is added to this mixture. After stirring for 2.5 h at 0°C, water is added, the product extracted with ether, dried over Na_2SO_4 and concentrated in vacuo, yielding the product as a white foam; $[\alpha]_D^{20}$ = - 61.6° (c = 0.5 in MeOH); MH^+ = 699.

Example 29-31

Boc-D-TMSal-Pro-NH-CH[$(CH_2)_2C_6H_5$]B-OPin

A. (+)-Pinanediol-(*S*)-1-chloro-3-phenyl-propane-1-boronate

3.47 g (30.0 mmol) Styrene and 3.6 g (30.0 mmol) catecholborane are stirred for 20 h at 100°C, yielding a yellow-brown oil. This oil is added to 5.0 g (30.0 mmol) (+)-pinanediol in THF and stirred overnight. After flash chromatography (8:2, hexane/EtOAc) (+)-pinanediol-2-phenyl-ethane-1-boronate is obtained.

Over a 20 min period 15 ml (24.0 mmol) BuLi (1.6 M in hexane) are added to a precooled (-100°C) solution of 2.1 ml CH_2Cl_2 and 37 ml THF. After stirring for 30 min at -100°C, 6.2 g (21.8 mmol) of the pinanediolboronate product obtained above in THF is added over a 20 min period. The reaction mixture is again stirred for 1 h at -100°C, after which 1.51 g (10.9 mmol) $ZnCl_2$ in THF is added. The reaction mixture is allowed to warm up to room temperature overnight. After concentration in vacuo the residue is dissolved in ether/H_2O. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. (+)-pinanediol-(*S*)-1-chloro-3-phenyl-propane-1-boronate is obtained as yellow-orange oil.

B. Boc-D-TMSal-Pro-NH-CH[$(CH_2)_2C_6H_5$]B-OPin

5.1 ml (8.09 mmol) Buthyllithium (1.6 M in hexane) is added to a precooled (-78°C) solution of 1.7 ml (8.09 mmol) hexamethyldisilazane in THF. The reaction mixture is stirred 1 h at room temperature, then cooled down to -78°C again. 2.68 g (8.09 mmol) (+)-pinanediol-(*S*)-1-chloro-3-phenyl-propane-1-boronate dissolved in THF is added. After stirring for 1 h at -78°C, the solution is warmed up overnight to room temperature. After recooling to -78°C 3 mol equivalents of HCl in dioxane are added. The mixture is stirred for 1 h at -78°C, then for 2 h at room temperature. After cooling down to -20°C, a solution of 3.7 g (8.09 mmol) of the active ester of Example 1, step B in CH_2Cl_2 is added, followed by the addition of 2.3 ml (18.2 mmol) triethylamine. The mixture is stirred for 1 h at -20°C and for 2 h at room temperature, then filtered. After concentration in vacuo, the residue is dissolved in ether/H_2O. The organic layer is dried

over Na_2SO_4 and concentrated in vacuo. After flash chromatography (6:4, hexane/EtOAc), the desired product is obtained; $[\alpha]_D^{20}$ = - 88.6° (c = 0.5 in MeOH); MH^+ = 654.

The Boc-D-TMSal-Pro-NH-CH[$(CH_2)_2$(*p*-Cl-C_6H_4)]B-OPin ($[\alpha]_D^{20}$ = - 83.2° (c = 0.5 in MeOH); MH^+ = 688) and Boc-D-TMSal-Pro-NH-CH[$(CH_2)_2$(*p*-NMe_2-C_6H_4]B-OPin ($[\alpha]_D^{20}$ = - 84.8° (c = 0.54 in MeOH); MH^+ = 697) products are obtained analogously.

Example 32

$CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHCHO]B-OPin

A. $CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-CH[$(CH_2)_4N_3$]B-OPin

5.15 g (8.00 mmol) of the azide product of Example 2, step C is added to 40.0 ml of acetic acid/conc. HCl (9:1) and stirred for 75 min. After concentration in vacuo, HCl·H-D-TMSal-Pro-NH-CH[$(CH_2)_4N_3$]B-OPin is crystallized from ether; mp: 87°C.

0.29 ml (2.40 mmol) pivaloyl chloride in THF is added to a mixture of 1.17 g (2.00 mmol) of the above abtained product and 0.34 ml (2.40 mmol) triethylamine in THF at 0°C and stirred for 1 h at this temperature. To this solution 533 mg (2.40 mmol) $CH_3[O(CH)_2]_3OCH_2CO_2H$ in THF and subsequently 4.0 ml of saturated aqueous $NaHSO_4$ are added. After stirring for 1 h at room temperature, saturated aqueous $NaHSO_4$ is added and the product is extracted several times with EtOAc. The organic layers are washed with 2 N H_2SO_4 and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product is dissolved in MeOH/water (6:4) and washed with hexane. After concentration of the MeOH/water layer, $CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-CH[$(CH_2)_4N_3$]B-OPin is obtained as an oil; $[\alpha]_D^{20}$ = - 74.5° (c = 0.83 in MeOH); MH^+ = 751.

B. $CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-CH[$(CH_2)_4NH_2$]B-OPin

1.05 g (1.40 mmol) of the product of Example 32, step A is dissolved in 14 ml EtOH and hydrogenated in the presence of 140 mg of 10% Pd/C and 0.84 ml (1.38 mmol) 2 N HCl. After 2.5 h, the catalyst is removed and water is added. The water layer is washed with ether and concentrated in vacuo, yielding 855 mg (80 %) $CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-$CH[(CH_2)_4NH_2]$B-OPin as an oil; $[\alpha]_D^{20}$ = - 74.2° (c = 0.61 in MeOH); MH^+ = 725.

C. $CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-$CH[(CH_2)_4NHCHO]$B-OPin

31 µl (0.80 mmol) formic acid and 71 µl (0.70 mmol) acetic anhydrid are stirred for 2 h at 50°C. After cooling to 0°C, 325 mg (0.43 mmol) of the borolysine product of Example 32, step B in THF and 0.12 ml (0.86 mmol) triethylamine is added. After stirring for 17 h at room temperature, EtOAc is added and the solution washed with 2 N H_2SO_4 and brine, dried over Na_2SO_4 and concentrated in vacuo. After RP-chromatography (MeOH/water, gradient 50% $H_2O \rightarrow$ 25 % H_2O), the desired product is obtained as an oil; $[\alpha]_D^{20}$ = - 80.8° (c = 0.27 in MeOH); MH^+ = 753.

Example 33

$CH_3[O(CH_2)_2]_3OCH_2C(O)$-D-TMSal-Pro-NH-$CH[(CH_2)_4NHC(O)NHEt]$B-OPin

325 mg (0.43 mmol) of the borolysine product of Example 32, step B in THF is cooled to 0°C and 40 µl (0.43 mmol) ethyl isocyanate and 48 µl (0.43 mmol) N-methyl morpholine is added. After 4 h stirring at room temperature, EtOAc is added and the solution washed with 1 N $NaHSO_4$, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in vacuo. After RP-chromatography (MeOH/water, gradient 50% $H_2O \rightarrow$ 25 % H_2O), the desired product is obtained as an oil; $[\alpha]_D^{20}$ = - 76.0° (c = 0.55 in MeOH); MH^+ = 796.

Example 34

H-D-TMSal-Pro-NH-$CH[(CH_2)_4NHCHO]B(OH)_2$

150 mg (0.231 mmol) of the formamide product of Example 2, step E is

dissolved in 2.5 ml acetic acid/conc. HCl (1:9) and stirred for 4 h at room temperature. After concentration in vacuo, the residue is dissolved 3 times in toluene and again concentrated in vacuo. The desired product is obtained as yellow foam; $[\alpha]_D^{20}$ = - 63.6° (c = 0.55 in MeOH); MH^+ = 415.

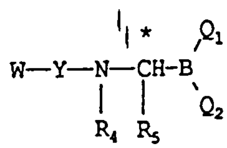
Example 35

H-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)NHEt]B$(OH)_2$

150 mg (0.217 mmol) of the Boc-D-TMSal-Pro-NH-CH[$(CH_2)_4$NHC(O)NHEt]B-OPin product of Example 21 is dissolved in 2.5 ml acetic acid/conc. HCl (1:9) and stirred for 4 h at room temperature. After concentration in vacuo, the residue is dissolved 3 times in toluene and again concentrated in vacuo. The desired product is obtained as orange foam; $[\alpha]_D^{20}$ = - 74.1° (c = 0.56 in MeOH); MH^+ = 458.

CLAIMS

1. A compound of formula I



I

wherein: W is hydrogen or an N-protecting group

Y is a sequence of n amino acids such that the n+1 amino acid peptide Y-Lys or Y-Arg has an affinity for the active site of a trypsin-like protease; where n is an integer of from 1 to 10 and in which at least one amino acid is an unnatural amino acid having a hydrophobic side chain;

Q_1 and Q_2 are the same or different and are selected from -OH, $-COR_1$, $-CONR_1R_2$, $-NR_1R_2$ or $-OR_3$ or Q_1 and Q_2 taken together form a diol residue;

R_1, R_2 and R_3 which may be the same or different, are C_{1-10}alkyl, C_{6-10}aryl, C_{6-10} aralkyl or phenyl substituted by up to three groups selected from C_{1-4}alkyl, halogen and C_{1-4}alkoxy;

R_4 is hydrogen or C_{1-10}alkyl

R_5 is a group -A-X

wherein A is $-(CH_2)_z-$ in which z is 2,3,4 or 5; $-CH(CH_3)-(CH_2)_2-$; $-CH_2-CH(CH_3)-CH_2-$; $-(CH_2)_2-CH(CH_3)-$; $-(CH_2)_2-C(CH_3)_2-$; $-CH(CH_3)-(CH_2)_3-$; $-CH_2-CH(CH_3)-(CH_2)_2-$; $-CH_2-CH_2-CH(CH_3)-CH_2$; $-(CH_2)_2C(CH_3)_2-CH_2-$; $(CH_2)_3-CH(CH_3)-$; $-(CH_2)_3-C(CH_3)_2$: $-(CH_2)_3CH(CH_3)CH_2-$; C_{6-10}aryl, C_{6-10}aralkyl

and wherein X is OH, SH, NR_6R_7 or phenyl,

wherein R_6 is H or C_{1-10}alkyl, and R_7 is C_{1-10}alkyl, $-CO-R_8$, $CS-R_8$, or SO_2-R_8

wherein R_8 is H or C_{1-10}alkyl, C_{1-10}alkoxy, C_{6-10}aryl, C_{6-10}aralkyl,

optionally substituted by up to three groups selected from halogen, OH, C_{1-4}alkoxy,

$C(NH)R_9$, $NR_{10}R_{11}$ and $C(O)OR_6$ (wherein R_6 is as defined above),

wherein R_9 is C_{1-3}alkyl or $N(CH_3)_2$ and wherein R_{10} and R_{11} (which may be the same or different) are H or C_{1-10}alkyl,

and the asymmetric carbon atom marked * may have the D- or L-configuration, and the compounds may comprise any mixture of these.

2. A compound according to claim 1 in which W is $H(CH_2CH_2O)_p$-, $R_{12}CO$-, $R_{13}OCO$- or $R_{14}SO_2$-, wherein:

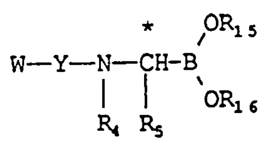
R_{12} = C_{1-6}alkyl or $H(CH_2\text{-}O\text{-}CH_2)_p$

R_{13} = C_{1-6}alkyl, phenyl, benzyl or naphthyl; and

R_{14} = phenyl, naphthyl or C_{1-4}alkylphenyl

wherein p = 3-30

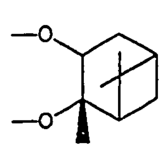
3. A compound according to claim 1 or 2 which is of formula Ia



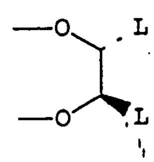
Ia

wherein: W,Y,R_4 and R_5 are as defined in claims 1 or 2 and R_{15} and R_{16} represent the residue of a dihydroxy compound.

4. A compound according to claim 1 or 2 wherein Q_1 and Q_2 together represent a group of formula (a) or (b)



a)



b)

in which L is a C_{1-4}alkyl group.

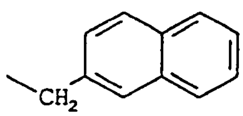
5. A compound according to any one of claims 1 to 4 wherein the unnatural amino acid is of formula II

$$\text{- NH - }\overset{R_{17}}{\overset{|}{CH}}\text{ - }\underset{\underset{O}{\|}}{C}\text{ -} \qquad \text{(II)}$$

wherein R_{17} is a hydrophobic group.

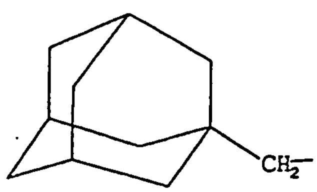
6. A compound according to claim 5 wherein R_{17} is R_{17}' and is a group of formula (c), (d), (e), (f), (g), (h) or (i)

c)



$—CH_2—Si(CH_3)_3$

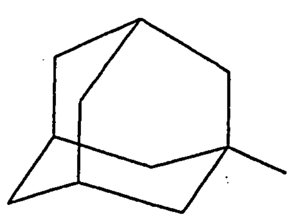
d)



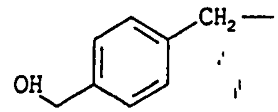
e)

$—CH_2—C(CH_3)_3$

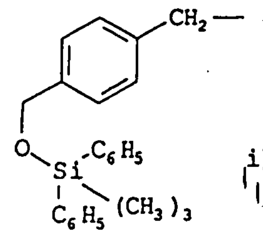
f)



g)



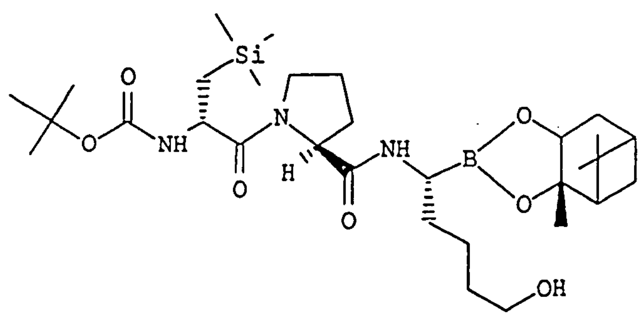
h)



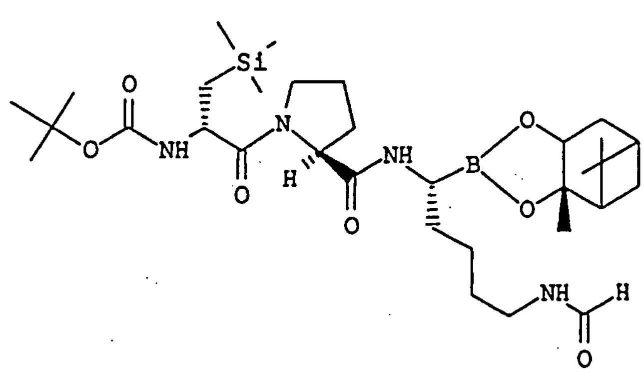
i)

7. A compound according to claim 1 wherein Y is a sequence of two amino acids of which the N-terminal amino acid is the unnatural amino acid and the other amino acid is L-proline.

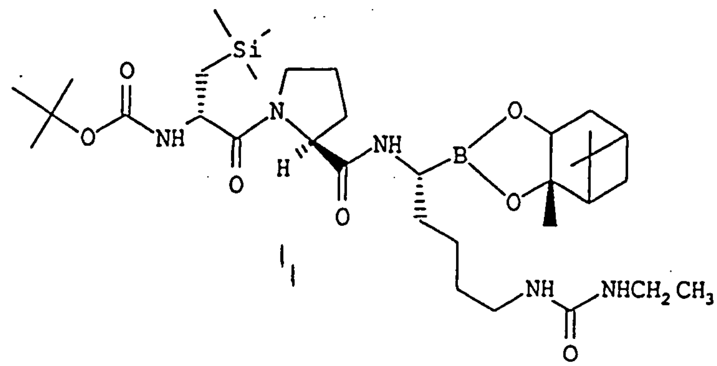
8. A compound according to claim 1 selected from the compounds of formulae III, IV and VI



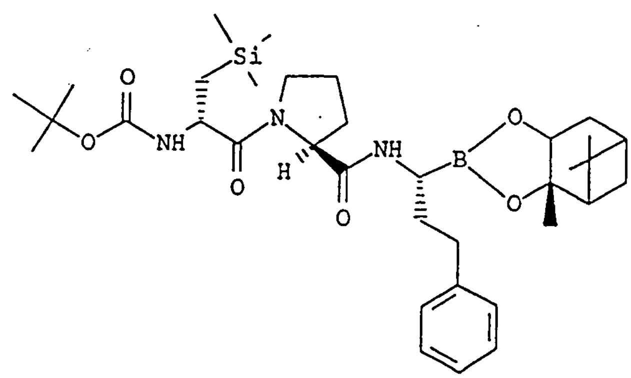
III



IV



V



VI

9. A process for the preparation of a compound of formula I as defined in claim 1 which comprises:

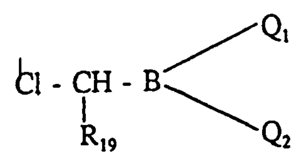
i) when X is NHCHO or NHCOalkyl, reacting a compound of formula I in which X is $-NH_2$ with an active form of the corresponding acid;

ii) when X is NHCOalkoxy, treating the compound of formula I in which X is $-NH_2$ with N, O -bis (trimethylsilyl) acetamide followed by addition of an active form of the corresponding ester;

iii) when X is NHC(S)NHalkyl, reacting the compound of formula I in which X is $-NH_2$ with the corresponding alkyl isothiocyanate;

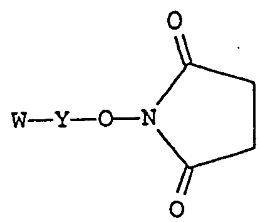
iv) when X is NHCHS, reacting the compound of formula I in which X is -NHCHO with Laweson reagent;

v) when X is optionally substituted phenyl, reacting a compound of formulae VII



VII

in which Q1 and Q2 are as defined in claim 1 and R_{19} is -A-phenyl (optionally substituted) wherein A is as defined in claim 1, with $LiN[Si(CH_3)_3]_2$, followed by hydrolysis with an excess of acid and coupling with a protected peptide of formula VIII



VIII

wherein W and Y are as defined in claim 1.

vi) when X is OH reacting a compound of formula I in which X is $-OSi(CH_3)_2C(CH_3)_3$ with a desilylating agent.

10. Use of a compound of any one of claims 1 to 8 as a inhibitor of trypsin like serine proteases.

11. Therapeutic composition containing a compound of any one of claims 1 to 8 together with pharmaceutically acceptable additives and/or diluents.

12. Use of therapeutic composition according to claim 11 for inhibition of trypsin like serine proteases.

13. Use according to claims 10 and 12 characterised in that trypsin-like serine

proteases are thrombin, factor X_a, kallikrein, plasma, prolyl endopeptidase and Ig AI protease.

14. Therapeutic composition having anti-coagulant or anti-thrombogenic activity containing a compound of any one of claims 1 to 8 together with pharmaceutically acceptable additives and/or diluents.

15. Use of a compound of any one of claims 1 to 8 as an inhibitor of thrombin.

16. Use of a compound of any one of claims 1 to 8 as an inhibitor of vascular remodelling following venous or arterial surgery or other forms of vascular damage.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 94/00595

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K5/06 C07K5/08 C07K5/10 C07K7/06 A61K37/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 471 651 (SANDOZ LTD.) 19 February 1992 see the whole document ---	1-16
A	EP,A,0 145 441 (E.I. DU PONT DE NEMOURS AND COMPANY) 19 June 1985 see claims; examples ---	1,10-16
A	EP,A,0 293 881 (E.I. DU PONT DE NEMOURS AND COMPANY) 7 December 1988 cited in the application see claims; examples -----	1,10-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search
20 June 1994

Date of mailing of the international search report
01-07-1994

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Authorized officer
Fuhr, C

INTERNATIONAL SEARCH REPORT	...ernational application No. PCT/EP 94/00595

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK : Although claims 10,12,13,15,16 as fas as they concern an in vivo treatment, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 94/00595

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0471651	19-02-92	AU-B-	643312	11-11-93
		AU-A-	8179291	20-02-92
		CA-A-	2048953	14-02-92
		JP-A-	4330094	18-11-92
		US-A-	5288707	22-02-94
EP-A-0145441	19-06-85	US-A-	4499082	12-02-85
		CA-A-	1253999	09-05-89
		DE-A-	3485568	16-04-92
		JP-A-	60146900	02-08-85
EP-A-0293881	07-12-88	US-A-	5187157	16-02-93
		AU-B-	623592	21-05-92
		AU-A-	1733288	08-12-88
		CA-A-	1328332	05-04-94
		DE-A-	3878991	15-04-93
		JP-A-	1063583	09-03-89
		US-A-	5242904	07-09-93
		US-A-	5250720	05-10-93